mittelbar nach der täglichen pH-Messung zu den Weibchen gegeben und auch dann, wenn keine Copula erfolgte, nach 30 Minuten wieder von diesen getrennt wurden. In Tab. 3 sind die 109 registrierten Copulae den unmittelbar zuvor gewonnenen pH-Werten zugeordnet. Dabei ergibt sich ein eindeutiger, statistisch hochsignifikanter Zusammenhang zwischen dem pH-Wert und der Zahl der Copulae, die im Bereich von 7,2—8,0 linear ansteigt.

Lipkow's Methode, den Erfolg der Copula mit Hilfe des Vagina-pH festzustellen (bei eingetretener Befruchtung ein gegenüber dem Dioestrus leicht erhöhtes Mittel von 7,6), ließ sich bei *Myopus* nicht anwenden, weil die zwischen dem 2. und 5. Tag nach der Copula erfolgte Schließung der Vagina Messungen unmöglich macht. Nach der Wiedereröffnung der Vagina schwankt der Scheiden-pH beim trächtigen Weibchen zwischen 7,0 und 7,5 (seltener 7,7), was genau dem Dioestrus-Niveau entspricht.

Vaginal-pH und Geschlechtsverhältnis der Nachkommen

Von den 32 untersuchten *Myopus*-Weibchen brachten 29 zusammen 75 Würfe mit 273 Jungen, von denen 82,4 % Weibchen waren. Wie in der Gesamtzucht schwankte der Weibchenanteil in der Nachkommenschaft der einzelnen Muttertiere zwischen 33,3 % und 100 %. Zwischen den unmittelbar vor der Copula gemessenen Scheiden-pH-Werten dieser Weibchen (n = 75) fanden sich jedoch keine entsprechenden Unterschiede. Wenn man diejenigen Weibchen, die ausschließlich weibliche Junge zur Welt brachten (n = 13), mit Kalela und Oksala (1966) als gesonderte Gruppe behandelt und denjenigen Muttertieren gegenüberstellt, die auch männliche Junge hatten (n = 16), ergeben sich für diese beiden Gruppen durchschnittliche Scheiden-pH-Werte von 7,92 und 7,85, die statistisch homogen sind. Übereinstimmend zeigte sich, daß Würfe mit hohem Männchenanteil ebenso wie Würfe, die ausschließlich Weibchen enthalten, sowohl bei hohem (8,0 und höher) als auch bei niedrigerem (7,5—7,7) Scheiden-pH konzipiert sein können.

Für die Endauswertung standen 56 Würfe mit 202 Jungen zur Verfügung, deren Konzeption gemessenen Scheiden-pH-Werten zugeordnet werden konnte (Tab. 4). Das bei den verschiedenen pH-Werten erhaltene und im Prozentsatz der Weibchen ausgedrückte Geschlechtsverhältnis der Nachkommen schwankt zwischen 75,9 % und 86,7 % (m = 78,4 %). Ganz abgesehen davon, daß diese Schwankung in gar keinem Verhältnis zu jener steht, die in der Nachkommenschaft der einzelnen Muttertiere ermittelt wurde (33,3 % bis 100 %), zeigen die vorliegenden Werte keine regelhafte Anordnung, sondern erweisen sich als rein zufallsbedingt und statistisch homo-

Tabelle 4

M. *schisticolor* (Lilljeborg) — Scheiden-pH des Muttertieres und Geschlechtsverhältnis der Nachkommen

(pH of the maternal vaginal fluid and sex ratio in the young)

Junge (young)	Copula bei Scheiden-pH von (copula at pH of)						total
	7,5	7,7	8,0	8,2	8,5	8,8	
n	28	58	101	3	3	9	202
♂	5	14	21	1	—	1	42
♀	23	44	80	2	3	8	160
♀ in %	82,2	75,9	79,2		86,7		78,4

gen, da nicht einmal der Unterschied zwischen den genannten Extrem-Werten signifikant ist.

Wenn das verfügbare Material auch nicht übermäßig groß ist, dürften die mehrfach abgesicherten und übereinstimmenden Befunde doch den eindeutigen Schluß zulassen, daß auch bei dem in dieser Beziehung besonders interessanten Waldlemming kein Zusammenhang zwischen dem Geschlechtsverhältnis der Nachkommen und dem Scheiden-pH des Muttertieres besteht.

Zusammenfassung

An 32 *Myopus*-Weibchen wurde kein Zusammenhang zwischen dem ungewöhnlichen 1:3 bis 1:4 Geschlechtsverhältnis dieser Art und der Wasserstoffionen-Konzentration in der Vagina des Muttertieres gefunden. Der Scheiden-pH liegt im Dioestrus bei 7,0—7,5, sinkt im Prooestrus auf 6,4—6,7 und steigt im Metoestrus auf 7,7—9,0. Die Copula findet durchweg während des pH-Maximums statt. Die danach an der Vagina konstatierbaren Veränderungen wurden hinsichtlich ihrer Brauchbarkeit als Trächtigkeits-Anzeiger beschrieben. Ein basaler Sexualzyklus mit annähernd konstanter Phasenlänge zeichnete sich allenfalls schwach ab. Biologisch wesentlicher ist offensichtlich, daß der Oestrus bei bestehender Fortpflanzungsbereitschaft jederzeit spontan durch die von einem brünstigen Männchen ausgehende Stimulation ausgelöst werden kann.

Summary

Sexual cycle, pH of vaginal fluid, and sex ratio in the palaearctic woodlemming, Myopus schisticolor (Lilljeborg)

It has been investigated on a material of 32 females if a causal connection exists between the extraordinary 1:3—1:4 sex ratio in the palaearctic woodlemming (Tab. 1) and the hydrogen ion concentration in the vaginal fluid. The latter ranges from pH 7.0—7.5 in diestrus, descends to 6.4—6.7 in proestrus and estrus, and ascends to 7.7—9.0 in metestrus. A cycle of a certain phase length appeared in outlines at best. It is obviously of more biological importance that the estrus can be stimulated by the appearance of an adult male at any time. Mating happens mostly during the maximum pH (Tab. 3). Normally, the vagina closes between the second and fifth day after conception and reopens between the eighth and twelfth day, soon after which it contains mucous and bloody secretions at diestrus-like pH values (Tab. 2).

As it is typical in this species the sex ratio in the young of the individual females ranged from 2:1 to 0:X (i. e. female descendants only). But there were no similar differences in the pH values of the maternal vaginal fluid. Correspondingly, litters containing a high portion of males as well as litters containing females only hat been conceived at high pH values as well as at lower ones. When the young of the investigated females were grouped according to the different pH values at which they had been conceived, no signifcant differences in the sex ratio of these groups resulted (Tab. 4). These corresponding results admit the conclusion that the exceptional sex ratio in *Myopus* has no connection with the hydrogen ion concentration in the maternal vaginal fluid.

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Biology of the rice rat *(Oryzomys palustris natator)* in a laboratory environment

VI. Differentiation of the sexes

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Introduction

The mean litter size of rice rats surviving to weaning in the Dundee colony was found to be 3.6 rats per litter (PARK and NOWOSIELSKI-SLEPOWRON 1972). Sex ratios within these litters, especially during the early phases when a number of young died from unknown causes or were eaten, proved difficult to assess accurately since any interference invariably stimulated tendencies of desertion or cannibalism. Since the numbers of any particular sex depend on the control exerted by a number of variables, only those young rats which survive can be usefully analysed. From the records of the colony, the males and females surviving weaning were found to be similar in number. A Chi-square test with correction for continuity showed that the sex distribution did not deviate significantly from 50:50 as P lay between 0.90 and 0.50.

The initial concept that sex ratios were under control of selection stemmed from DARWIN (1871), and this was followed by a more intricate idea — normally acceptable to-day as the theory of evolution of sex-ratios (FISHER 1929). The theory was based on the point that because each sex contributes equally to the genetic composition of future generations, selection in turn, would act to equalise the expenditure of energy in producing offspring of each sex. This meant that the population sex ratio should tilt towards equality if the requirements of producing males and females were equal. Further work by KOLMAN (1960) showed that equal expenditure of energy in the production of males and females did not always mean that the sex ratio emerged equal. Another idea (MACARTHUR 1965) was that the sex ratio would result from the full extent of the product of the numbers of each sex successfully raised. Recently (LEIGH 1970) it was shown that differential adult

mortality between the sexes did not change the expected sex ratio at birth. The sum total of these approaches is that the most important factor influencing the population sex ratio should be the total amount of energy used by the parents on all young from conception to the end of parental duties.

The sex ratio of most rodents is generally quoted as being approximately 1:1, and work on an open and closed vole population (MYERS and KREBS 1971) returned a similar figure. Variation in sex ratio may have affects on population regulation and the evolutionary theory. The changes in environmental conditions being undergone by the rice rats in an laboratory environment will, in time, have considerable influence on the selection factors. As rats within the preweaning phase of development lie at one end of the scale, the sexes can be examined and differentiated by visual characteristics and the instantaneous relative growth rates of BRODY (1945) as an indication of the effect which may arise following weaning. The use of the differential growth rate as a measure is important since the rice rats examined during the preweaning phase in the present material were originally arranged in sexes of equal numbers and investigated as a 24-hour series instead of a longitudinal study with attendant survival failures and insufficient data.

Material and methods

Specimen were obtained from a 24-h series of rice rats *(Oryzomys palustris natator)* aged from birth to 21 days. Birth of the rats was arbitrarily accepted as 1.00 am., unless there were indications of a recent birth upon which adjustments were accordingly made. Other births were noted as they occurred. The number of rats used within each 24-h stage 6—8 with equal division of the sexes. Due to a low birth rate and high mortality the rats forming each stage were drawn from a number of litters.

The rats were killed by an overdose of ether and weighed immediately. Measurements of the anogenital distance were obtained after weighing before material for other studies was removed. Calculations were based on the arithmetic mean of the observed weights and anogenital distances of the male and female rats of the series.

The equation for instantaneous relative growth (BRODY 1945) is written as

$$k = \frac{\ln W_2 - \ln W_I}{t_2 - t_I} \tag{1}$$

where: k = instantaneous relative growth rate; $\ln W$ — natural logarithm of weight W at time t.

Modification of the instantaneous relative growth equation to give a ratio comparison for male and female was introduced by PARK (1970) and is included below for clarification.

The equation for the male can be written as:

$$k_M = \frac{\log M_2 - \log M_I}{t_2 - t_I} \tag{2}$$

M representing the male weight.

Similarly the equation for the female follows thus:

$$k_F = \frac{\log F_2 - \log F_I}{t_2 - t_I} \tag{3}$$

F representing the female weight.

By incorporation of equations (2) and (3), the time factor is thus eliminated:

$$\frac{k_M}{k_F} = \frac{\log M_2 - \log M_I}{\log F_2 - \log F_I} \tag{4}$$

In short, k_M/k_F is based on unity so that if the result is greater, the growth rate of the male is faster, whereas if less, then the female growth rate is faster.
The equation is only valid if the time interval used is identical for both.

Observations

Within the preweaning stage of development the division of the sexes of young rats can be distinguished by means of external characters — basically those used by Jackson (1912) on the albino laboratory rat *(Rattus norvegicus)*. The male rice rat is characterised from birth by a larger genital papilla and a greater distance between anus and genital papilla than the female rice rat.

The spectrum of change in the anogenital distances of male and female rice rats over the first 21 days of postnatal development is shown in Table 1 and has been arranged alongside those of the albino laboratory rat (Sprague-Dawley strain) as a measure of species difference. The anogenital distance remains the only characteristic for identification which is present throughout the full 21 days since the appearance of the eight pairs of mammary glands in the female rice rat only occurs after some 7—8 days following birth.

External measurements of young rice rats of both length and weight did not appear to produce any significant sex differences but were analysed for confirmation. Calculations of the instantaneous relative growth rat constant k, are summarised in Tables 2 and 3. The results cover the period of preweaning growth by dividing it into two separate series based on odd and even-day intervals. The first two columns of the tables are calculated from the mean weights of the males and females at each particular stage. The last two columns represent the calculations derived from the instantaneous relative growth rate ratio k_M/k_F and the letter indicating the trend. Since the tables represent two separate series, based on two-day intervals, and therefore overlap one another over the 21 day growth phase being examined, each table must be interpretated within its own format. The validity of the results are based on the particular group of selected time intervals to which it belongs i. e. odd or even-day intervals. Although trends indicated in the final columns lean towards greater female growth in the second part of the preweaning phase, it is possible that this lies well within sampling error.

Regression lines were formulated for both weight and head-body length of the rice rats (Figs. 1 and 2) and these supported the contention

Table 1

Sex Differentiation
by the Anogenital Distance – Preweaning Rats

Age	Albino rats		Rice rats	
	Male	Female	Male	Female
0	2.4	1.2	2.0	0.9
1	2.6	1.4	2.3	1.1
2	2.9	1.7	2.7	1.3
3	3.2	2.1	3.0	1.6
4	3.7	2.4	3.4	1.9
5	4.3	2.6	3.9	2.0
6	4.8	2.7	4.3	2.2
7	5.2	2.9	4.7	2.6
8	5.5	3.0	4.9	2.9
9	5.9	3.3	5.3	3.0
10	6.4	3.7	5.7	3.2
11	6.9	3.9	6.0	3.6
12	7.2	4.2	6.4	3.9
13	7.6	4.5	6.9	4.0
14	7.9	4.8	7.3	4.4
15	8.2	5.0	7.8	4.9
16	8.7	5.4	8.0	5.2
17	9.2	5.9	8.4	5.4
18	9.9	6.4	8.9	5.8
19	10.5	6.9	9.3	6.0
20	11.1	7.0	9.8	6.2
21	11.6	7.3	10.1	6.4

Age in days; measurements in mm (arithmetic mean).

Table 2
Instantaneous relative growth rates of rice rats
Odd Day Intervals

Age	Male	Female	kM/kF	Trend
1	0.3168	0.2790	1.14	M
3	0.0651	0.1367	0.48	F
5	0.3387	0.2731	1.24	M
7	0.0444	0.0103	4.31	M
9	0.0246	0.0278	0.88	F
11	0.2047	0.2120	0.97	F
13	0.0733	0.1209	0.61	F
15	− 0.0648	− 0.1230	0.53	F
17	0.1728	0.1933	0.89	F
19	− 0.0040	− 0.0243	0.16	F

M = male, F = female

Table 3
Instantaneous relative growth rates of rice rats
Even Day Intervals

Age	Male	Female	kM/kF	Trend
2	0.1769	0.2551	0.69	F
4	0.3193	0.0592	5.39	M
6	− 0.0991	0.1116	− 0.89	F
8	0.2324	0.1915	1.21	M
10	0.1396	0.0896	1.56	M
12	− 0.0747	0.0275	− 2.72	M
14	− 0.0027	− 0.0297	0.09	F
16	0.1882	0.2886	0.65	F
18	− 0.0456	− 0.1583	0.29	F
20	0.1047	0.1267	0.83	F

M = male, F = female

that sex differentiation was not significant and that for purposes of analysis the data of male and female rice rats within the first 21 days of development could therefore be pooled.

Eruption of the crowns of the molar teeth through the oral mucosa of both sexes was noted. No asymmetry of eruption between the right and left sides was detected in the first and second maxillary and mandibular molars although there were some signs of instances of asymmetry occurring in the third molars. Tooth eruption, although variable, did not show any tendency towards sex differences and those which do exist cannot be regarded as of significance.

Discussion

Predictability of sex by morphological characteristics does not incur many errors once the particular characteristics have assumed a recognisable form. This approach was followed by JACKSON (1912) and concerned three morphological characters; the anogenital distance in which the distance between the anus and the genital papilla was found to be greater in the male than in the female of the same age; the size of the genital papilla and, finally, the presence of mammae. A fourth characteristic was suggested in which a small area lying ventral to the anus of the male remained devoid of hair. Only the first two characteristics are applicable to all age groups.

Apart from morphological aspects, the criterion of sex difference of animals following weaning has been based on body size since the males are usually larger, heavier, and have greater bone weight and muscular development than the females. This rule, however, does have exceptions in the hamster and rabbit where the female is often larger. ZAWADOWSKY (1931) showed that size in chickens stemmed from the genetic constitution rather than sex hormone secretion. Sex hormones do exert an important influence since if a male ferret — normally twice the size of the female — is castrated at birth, it will then grow to the size of the female. In the rice rats the sex difference marked by size and weight was found to gradually emerge after weaning until firmly established by 1 month.

Within the preweaning phase of development, the use of weight as a way of separating the sexes was used by KING (1915), and males were noted as being heavier at birth. This particular point was supported by ANGERVALL (1959) while in the same year, ACHESON, MACINTYRE and OLDHAM reported that male rats were heavier than females near the end of the preweaning period. Contradictory findings have been reported by LINDH (1961) during an investigation on the prenatal weight of rats and by SWANSON and WERF ten BOSCH (1963), the consensus of opinion favoured a lack of significant evidence of sex difference by weight. These findings have been confirmed in previous work on the albino laboratory rat (PARK 1970). HUGHS and TANNER (1970) studied the longitudinal growth of black-hooded rats
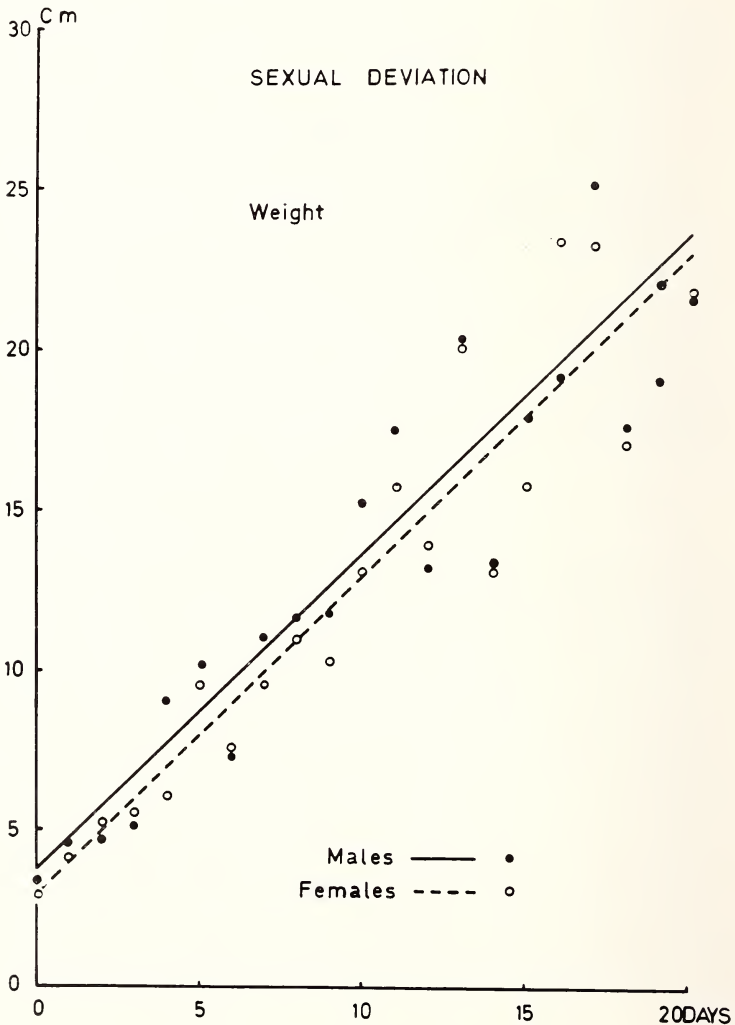

Fig. 1. Regression line analysis of the mean weights of male and female rice rats ranging from birth to 20 days and owing that no significant sexual deviation exists
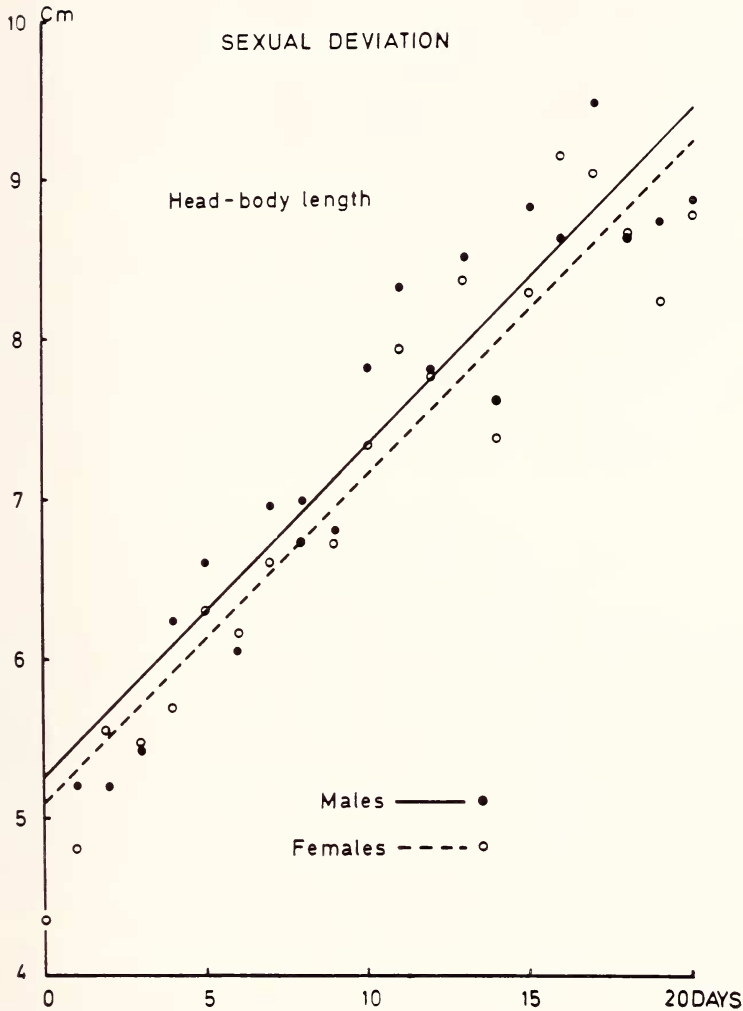
Oryzomys palustris natator.

SEXUAL DEVIATION

Head - body length

Males ———— •
Females – – – – ○

Fig. 2. Regression line analysis of mean head-body lengths of male and female rice rats ranging from birth to 20 days and showing that no significant sexual deviation exists

and although the males were marginally larger than the females, the statistical significance only emerged after weaning had occurred. Another longitudinal study — but with emphasis on molar eruption — was undertaken by KÖNIG and MARTHALER (1958, 1960). They examined Osborne-Mendel and Sprague-Dawley strains of albino rats and correlated growth (weight) with the sequence of molar tooth eruption in males and females. Differences due to sex were not found in the Osborne-Mendel rats for either molar eruption or growth. In the Sprague-Dawley strain, however, there were some weight differences during the later stages but no eruption differences. SWANSON and WERF ten BOSCH (1963) also noted that some of the males were slightly heavier than the females. Difference in weight between

the sexes of pre-weaning rats is, at the best, small in magnitude and can therefore be regarded as part of the variation existing between and within litters and which stems from the genetic endowment and the maternal environment.

Sex differentiation of the rice rats based on morphological characters has been clearly shown by the anogenital distances (Table 1) and by the emergence of the mammary glands. The external measurements (weight and length) were examined and calculations using weight — the instantaneous relative growth rate and the k ratios (Tables 1 and 2) failed to give a satisfactory difference although a slight trend towards the females appeared near the end of the preweaning phase. Finally, the regression line analysis (Figs. 1 and 2) showed that for purposes of investigation that all the males and females could be pooled during the first 21 days of postnatal development. Patterns of molar tooth eruption in the rice rat did not indicate any sex differences.

The complexity and sensitivity of the responsiveness of various species members to environmental manipulations have emerged from the work of SCHNEIRA (1959) and RHEINGOLD (1963) who investigated the relationship between certain early experiences and physiology together with the nature of maternal and infant interactions. An example of environmental manipulation is the culling of litters so that they are formed only of one sex — a practice used for studies involving castration or gonadal hormone administration.

The significance of these procedures, as related to sex, was first noted by BRAIN and GRIFFIN (1970) who, during analysis of control litters, found that the body weights taken at weaning showed that males and females in litters consisting of littermates of the same sex were heavier than males and females, respectively, in litters of equal size but of littermates of both sexes. These differences were observed to gradually level off over the next few weeks. This recovery phenomenon appears to follow the criteria outlined in the "Principle of equifinality" (BERTALANFFY 1960). This particular principle deals with the accepted biological fact that after a temporary cessation of growth, or initial differences in size, many animals reach a final size characteristic of species. Since the preliminary observation suggested a relationship between the type or amount of behavioural interactions within a litter and the sex of the members in the litter, further work was undertaken by BRAIN and GRIFFIN (1970). They formulated three types of litters; a homogeneous-male litter, a homogeneous-female litter, and finally, litters with equal numbers of males and females.

Examination of the results showed that males and females reared in litters formed only of members of the same sex were heavier than males and females, respectively, brought up in litters of equal size but containing both sexes. It is interesting to note that the differences were evident at 14 days. In general terms, it is well known from personal observation and from reports of other workers, including the recent paper by HUGHS and TANNER (1970), that there are many instances where males are fractionally larger than females at birth and thus maintain a slight advantage, although not at a significant level, throughout the course of the preweaning phase. In addition to this size (and weight) difference are strong complicating factors involving the genetic endowment and the external (maternal) environment. Basically, the rice litters varied considerably in size — rats numbering from 1 — 6 and since the number required at each age group was 6 (3 males and 3 females) the final composition of an age stage could rest on rats arising from as many as 4 different litters. Males and females often being reared singly or in groups, while others were reared of both sexes, but in uneven numbers.

Obviously, throughout the collection of rice rats for the various stages within the preweaning phase of development, the results noted by BRAIN and GRIFFIN